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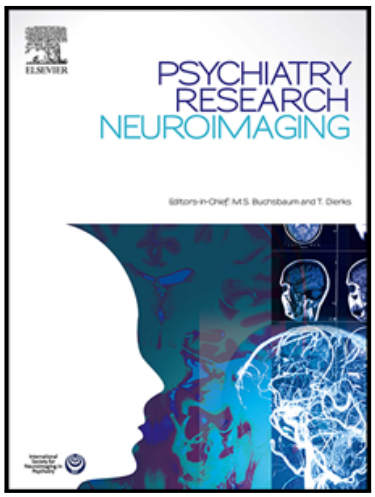

Systematic review of in-vivo neuro Magnetic Resonance Spectroscopy for the assessment of Posttraumatic Stress Disorder

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Highlights

- Reduced N-Acetylaspartate (NAA) in multiple regions, in posttraumatic stress disorder.
- Reductions in NAA are Irrespective of atrophic change in the majority of studies.
- Recent studies have found changes in excitatory and inhibitory neurotransmitters.

Systematic review of *in-vivo* neuro Magnetic Resonance Spectroscopy for the assessment of Posttraumatic Stress Disorder

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Abstract:

Posttraumatic stress disorder (PTSD) is a trauma and stressor-related disorder that results in complex somatic, cognitive, affective and behavioural effects, after exposure to traumatic event(s). Conventional imaging (T1 and T2 weighted magnetic resonance imaging) has little to offer in the way of diagnosis of mental health conditions such as PTSD and there is currently no objective diagnostic test available. Magnetic resonance spectroscopy (MRS) allows for non-invasive measurement of metabolites and neurochemicals in the brain using a conventional MRI scanner and offers the potential to predict, diagnose and monitor PTSD. This systematic review summarises the results of 24 MRS studies, performed between 1998 and 2017, to measure neurochemical differences, occurring as a consequence of PTSD. The most consistent finding in subjects with PTSD is lower N-acetylaspartate levels in the hippocampus and anterior cingulate cortex, with and without atrophic change. More recent studies, using more advanced techniques and modern hardware, have shown evidence of glutamatergic dysfunction and differences in gamma-aminobutyric acid levels in the brain of patients with PTSD. Conflicting results have been reported in choline-containing metabolites and there is emerging evidence of glutathione being affected. Myo-inositol and creatine are unchanged in the majority of studies.

1 Introduction

Posttraumatic stress disorder (PTSD) is a trauma and stressor-related disorder that results in complex somatic, cognitive, affective and behavioural effects, after exposure to traumatic event(s). PTSD is precipitated by a number of factors, including exposure to actual or threatened death or serious injury or a response to intense fear, helplessness or horror. PTSD is characterised by persistent re-experiencing of the traumatic event; avoidance of stimuli related to the trauma; and hyperarousal for at least one month post trauma (American Psychiatric Association, 1994), leading to significant psychosocial impairment for patients. PTSD is common in the general and military populations with an estimated 12-month prevalence of 5.2% vs. 8.3%, respectively (McFarlane et al., 2010).

In vivo neuro proton magnetic resonance spectroscopy (MRS) provides a non-invasive measurement of metabolites in the brain using a conventional magnetic resonance imaging (MRI) scanner at a field strength of 1.5 or 3T. In vivo MRS can measure differences in parenchymal metabolism in both the healthy and diseased brain (Mountford et al., 2010) and has the potential to provide biomarkers for psychiatric disease, which often has no characteristic findings on conventional T1 and T2 weighted structural MRI (Foerster et al., 2012; Murray et al., 2014). Up to 35 signals from metabolites, lipids, and macro-molecules can be measured using one dimensional (1D) neuro MRS at 3T (Provencher, 2001). Commonly used 1D MRS pulse sequences, include Point-RESolved Spectroscopy (PRESS) and Stimulated Echo Acquisition Mode (STEAM). Shown below in Figure 1 is a typical 1D neuro MRS spectrum (3T using a 64-channel head coil) from a healthy human brain labelled with the most commonly measured metabolites. MRS has the potential to shed further information on the pathogenesis and mechanisms behind PTSD, particularly when state of the art scanners are employed, which measure neurotransmitters such as gamma-aminobutyric acid (GABA), aspartate (Asp) and glutamate (Glu) non-invasively. This will further advance knowledge gained from other neuroimaging techniques such as functional magnetic resonance imaging (fMRI) and diffusion-weighted imaging (DWI). Additionally, in vivo neuro MRS is a promising technique that may help to predict patients at risk for PTSD and to non-invasively diagnose and monitor PTSD.

In this review, we synthesize previous work utilising proton MRS to characterise metabolic differences in the brain of patients with PTSD. Also, we give researchers a broader background on key MRS metabolites and brain regions implicated in the pathogenesis of PTSD that will help inform research findings and conclusions. Finally, we aim to determine if certain metabolites correlate with clinical variables, to identify possible MRS biomarkers of disease severity. We conclude with a discussion on the limitations and outstanding challenges facing researchers, utilising MRS to investigate PTSD.

1.1 Brain Regions Implicated in PTSD

Multiple brain regions, specifically, the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), amygdala, hippocampus and insular cortex have been implicated in the

pathogenesis of PTSD from imaging studies using positron emission tomography (PET), fMRI and structural MRI (Etkin and Wager, 2007; Lanius and Olff, 2017).

1.1.1 Hippocampus

The hippocampus is anatomically and functionally closely related to the amygdala; both structures play a significant role in the fear, fear extinction and anxiety networks, along with the hypothalamic-pituitary axis (Tovote et al., 2015), making the hippocampus an ideal region to interrogate with MRS.

Initially, animal models were used to identify a relationship between stress and morphological change of the hippocampus. This damage may be the result of Glu toxicity, prolonged exposure to elevated glucocorticoids or differences in brain derived neurotrophic factors or a combination of all these factors (Magarinos et al., 1996; McEwen, 2000; McEwen et al., 1992; Sapolsky, 1996; Sunanda et al., 1995).

Several volumetric meta-analyses have identified a reduction in hippocampal volume (Kitayama et al., 2005; Smith, 2005). Most recently O'Doherty et al. (2015) reviewed 36 studies quantifying human hippocampal volume and found smaller volumes bilaterally in PTSD subjects, with a greater reduction in the left hippocampus. It is still unclear if hippocampal atrophy is congenital or acquired in PTSD; however, twin studies have provided early evidence that lower hippocampal volume may be a pre-existing risk factor for PTSD (Gilbertson et al., 2002). Spectroscopy has the potential to provide complementary, non-invasive metabolic measurement of the MR visible chemistry and neuronal integrity of the hippocampus and may identify these differences prior to structural MRI changes (Schuff et al., 2001). Currently, there are no prospective MRS studies that have aimed to determine if metabolic differences precede atrophy in PTSD.

1.1.2 Amygdala

The amygdala appears to play a key role in the pathogenesis and expression of PTSD. Fear conditioning relies on an interplay between the amygdala and the prelimbic cortex of the mPFC (Bauer, 2016). The amygdala is responsible for the association of conditioned and unconditioned stimuli and the mPFC is involved in the expression of fear memory (Alden et

al., 1994; Amorapanth et al., 2000). The amygdala is also critical in the extinction of fear memories, thought to occur within the basolateral amygdala (Amano et al., 2011).

Functional MRI studies have identified increased activity within the amygdala in PTSD (Hughes and Shin, 2011). However, volumetric differences within the amygdala have been less clear cut; a recent meta-analysis concluded that there was no significant reduction in amygdala volume when trauma exposed controls were compared to PTSD subjects (O'Doherty et al., 2015). Yet the same meta-analysis identified smaller amygdala volumes bilaterally in PTSD subjects when compared to healthy controls (O'Doherty et al., 2015).

There are technical challenges of acquiring spectroscopy of the amygdala due to its small size, measuring 1.2cm^3 on average (Brabec et al., 2010). There are currently no MRS studies published on neurochemical differences in the amygdala in PTSD. However, one group has shown it is technically feasible to acquire MRS data from the amygdala (Nacewicz et al., 2012). There are now clinical research scanners at 3T that may well allow MRS of this region to be evaluated.

1.1.3 Medial Pre-Frontal Cortex

The mPFC refers to the medial anterior frontal lobe parenchyma, a region with bidirectional white matter connections with the amygdala (Pape and Pare, 2010). The mPFC consist of two subregions: the prelimbic cortex (PL) and infralimbic cortex (IL), that in the rodent brain exert dual control over the amygdala, with the IL supporting fear expression and the PL supporting fear extinction. The human mPFC also contains the ACC, as shown in Figure 2. The human dorsal anterior cingulate cortex (dACC) is thought to be a functional homologue of the rodent PL (Milad and Quirk, 2012). The IL has been implicated in the expression of learned but not innate fear (Sierra-Mercado et al., 2011; Vidal-Gonzalez et al., 2006), and in humans the ventromedial pre-frontal cortex (vmPFC) is thought to function as the homologue of the IL (Milad and Quirk, 2012). Additionally, the mPFC appears to play a role in the cognitive processing of emotional tasks (Phan et al., 2004), integrating affective stimuli received via inputs from the amygdala and hippocampus and evaluating these inputs against previous experience and behavioural goals (Lanius and Olff, 2017).

1.1.4 Anterior Cingulate Cortex

The cingulate cortex is located in the centre of the brain, anterior to the splenium of the corpus callosum. The anterior component of the cingulate cortex is subdivided into the dorsal, ventral and rostral components and two of these regions are shown in Figure 2. The dACC has been implicated in the expression and acquisition of conditioned fear responses (Milad et al., 2007).

Higher resting state fMRI (rs-fMRI) and task-based activity (non-emotional tasks) has been identified in the dACC in PTSD patients and their non-traumatised twins (Shin et al., 2009), suggesting that higher rs-fMRI activity within the dACC may predispose for the development of PTSD.

The rostral anterior cingulate cortex (rACC) has also been implicated in the pathogenesis of PTSD, where it has been shown to be hypoactive in fMRI studies, resulting in reduced amygdala inhibition (Shin and Liberzon 2010, Admon, Milad et al. 2013). Like the hippocampus, the ACC has been shown to have smaller volumes bilaterally in PTSD when compared to controls and these differences are thought to be acquired as a result of the disease (Kasai et al., 2008; Kitayama et al., 2006). There is also some evidence that the cortical thickness of the dACC correlates with the magnitude of a conditioned fear response (Hartley et al., 2011).

1.1.5 Insula Cortex

The insula cortex is located within the centre of the cerebral hemispheres and is extensively interconnected to regions, including the primary and secondary sensory cortex, anterior cingulate cortex, hippocampus, amygdala and the autonomic nervous system (Augustine, 1996). The insula cortex has been associated with interoceptive awareness of negative emotion such as anticipatory anxiety of guilt (Phan et al., 2004) and has been shown to activate in response to fearful facial expressions (Calder et al., 2001) and fearful conditional stimuli (Sehlmeyer et al., 2009). Therefore, the insula cortex is well positioned to convey somatic sensations, displayed as higher autonomic activation, elicited by interoceptive negative emotion. In the meta-analysis undertaken by Etkin and Wager (2007), the insula and amygdala demonstrated higher activation in PTSD, a finding also noted in social anxiety disorder and specific phobia disorders, suggesting a common mechanism. Few studies have

investigated volumetric differences in the insula, however, two studies have identified lower gray matter density in PTSD subjects (Kasai et al., 2008; Nardo et al., 2010) and one study performed in twins, suggested the abnormality may be acquired in PTSD.

1.2 Important brain metabolites

Below, a brief introduction to the metabolites that have been implicated in PTSD is given.

1.2.1 N-Acetylaspartate (NAA)

NAA is an amino acid derivative present in the brain in high concentrations and provides the most intense singlet resonance at 2.01 ppm, in a proton MRS spectrum acquired from a healthy brain (Luyten and den Hollander, 1986; Moffett et al., 2007; Tallan, 1957; Tallan et al., 1956). The resonance is shouldered by the N-actelyaspartylglutamate (NAAG) singlet peak (Govindaraju et al., 2000).

The function of NAA is an ongoing area of research, however, it is thought to function as an organic osmolyte. As an immediate precursor of NAAG, NAA acts as a source of acetate and facilitates energy metabolism in neuronal mitochondria (Moffett et al. 2007).

Reduced NAA was first thought to indicate an irreversible loss of neuronal density. However, a decrease in NAA has been shown to be reversible in conditions such as acute brain injury or methamphetamine abuse (Maddock and Buonocore, 2012) and is thought to represent reversible neuronal or mitochondrial dysfunction (Moffett et al., 2007). Therefore, the proton MRS signal from NAA indicates the viability, health and density of neurons and therefore can indicate neuronal mitochondria dysfunction (Moffett et al., 2007).

1.3 Glutamate and Glutamine

Glutamate functions mainly as an excitatory neurotransmitter and is the most abundant amino acid in the human brain (6-13mmol kg_{ww}^{-1}) (Govindaraju et al., 2000; Ramadan et al., 2013). The resonances of Glu and Gln are in the region of 3.74 – 3.75 ppm and 2.04-2.45 ppm and are overlapped by Gln, GABA and NAA (Govindaraju et al., 2000). The chemical structures of Glu and Gln are very similar resulting in significant overlap in the spectra collected at 1.5, 3T and 4T but not at 7T (Terpstra et al., 2016), and for this reason the composite resonances are referred to as Glx at lower field strengths.

Gln is a precursor for the excitatory neurotransmitter Glu and is located within astrocytes (Govindaraju et al., 2000; Lin et al., 2015). The primary role of Gln is to act as the 'storage form' of amino acid neurotransmitters such as Glu and GABA.

1.3.1 GABA

GABA is the primary inhibitory neurotransmitter in the brain. It contains three methylene (CH_2) groups that produce a complex one-dimensional spectrum with resonance multiplets centred at 1.89, 2.28 and 3.01 ppm. There is considerable overlap with resonances of NAA, Glu and creatine (Cr), which makes GABA difficult to reliably distinguish using conventional one-dimensional spectroscopy at 1.5 and 3T field strengths (Maddock and Buonocore 2012). It is important to remember that in a one dimensional MR spectrum the resonances are overlapping so the resultant difference may be due to other contributing molecules. Specialised pulse sequences are regarded as accurate ways to quantify GABA, the most common of which is MEGA-PRESS J–difference editing sequence (Mescher, Merkle et al. 1998) and 2D JPRESS (Jensen et al., 2009). Alternatively, GABA can be measured in conventional short echo time (TE) MRS using a fitting algorithm, such as the one implemented in LCMODEL (Provencher, 1993).

1.3.2 Myo-inositol (mI)

Myo-inositol is the most abundant form of inositol found in the brain. Using proton MRS, mI has a prominent mutiplet peak at 3.52 and 3.61 ppm (Govindaraju, Young et al. 2000). It is thought to be a astroglial marker (Brand et al., 1993).

1.3.3 Choline (Cho)

Choline is a vital constituent of many phosphoglycolipids, present within cell membranes (Koolman and Röhm, 2013), and contains contributions from glycerophosphorylcholine and phosphorylcholine. An increase in glycerophosphorylcholine and/or phosphorylcholine can indicate an increase in synthesis or breakdown of membrane phospholipids and results in a subsequent increase in the 3.21 ppm peak on proton MRS (Boulanger et al., 2000; Geddes et al., 1997).

1.3.4 Total Creatine (tCr)

Total Cr gives rise to the second largest peak in a typical brain MR spectrum at 3.03 ppm and contains contributions from Cr and phosphocreatine (PCr). PCr is a precursor for ATP and therefore tCr is thought of as an in vivo mitochondrial energy marker. tCr has been shown to be relatively stable in the healthy brain, with no significant daily intrasubject variation and for that reason is often used as an internal reference for other metabolites (Saunders et al., 1999; Soreni et al., 2006). However, tCr been shown to be disturbed in pathologies that alter cell metabolism, such as stroke and malignancy (Howe et al., 2003; Mathews et al., 1995) and is therefore used with caution as an internal reference metabolite.

2 Method

2.1 Literature search and inclusion criteria

A literature search was conducted using PubMed and PsychNet in April 2018 using the following keywords: (i) “post-traumatic stress disorder” and (ii) “PTSD” combined with the following subcategory keywords: (iii) “magnetic resonance spectroscopy” and (iv) “MRS”. Studies were selected if they satisfied the following conditions: in vivo human MRS studies, the patient group had a Diagnostic and Statistical Manual (DSM) based diagnosis of PTSD, were written in English and were compared with either a healthy or traumatised control group that did not have PTSD. None of the studies included participants with severe traumatic brain injury. Where any two or more studies included the same or overlapping patient populations, only the study with the largest sample size was included.

3 Results

A total of 27 studies were identified. Three studies were excluded: the study by De Bellis et al. (2001) was excluded as it was a case study with a single participant; the investigation performed by Henigsberg et al. (2011) as it was a therapeutic study and Neylan et al.’s (Neylan et al., 2003) study as it contained overlapping populations with Schuff et al.’s (Schuff et al., 2001) study. The remaining 24 studies were published between 1998 and 2017 and are summarised below in Table 1. Metabolic findings relevant to PTSD are shown below.

3.1 N-Acetylaspartate

The most consistent MRS finding identified in PTSD was a reduction in NAA.

Fifteen studies investigated NAA levels in the hippocampus. Several early studies refer to the to the hippocampus as the medial temporal, as the MRS voxel contained the hippocampus medial temporal lobe parenchyma. Six studies used absolute quantification (studies 3, 5, 13, 14, 16, and 24) and half of these found no significant difference in NAA (studies 5, 14 and 16). The positive studies found a statistically significant reduction in NAA levels in both hippocampi. Study 5 found a trend toward lower NAA in the hippocampus ($p = 0.054$), however, the sample was small and this may have limited its statistical power.

Eleven studies (1, 4, 6, 8, 9, 11, 12, 13, 14, 15 and 17) used Cr as an internal reference (some used both absolute and relative quantification). Four studies (6, 8, 12 and 13) identified no difference in the hippocampal NAA/Cr. One study (1) found higher NAA/Cr in the right hippocampus only, two studies (4 and 11) found lower NAA/Cr in the left hippocampus only and the remaining studies (9, 14, 15 and 17) found a significant reduction in NAA/Cr in both hippocampi.

Several early studies hypothesised that reductions in NAA may precede morphological change in the hippocampus. Many studies (3, 9, 12, 14, 16, and 24) found no significant difference in hippocampal volume, while the majority of studies (1, 4, 5, 6, 8, 12, 13 and 15) did not quantify the hippocampal volume. Shu et al. (2013a) identified a significant reduction in the normalised left hippocampus along with a significant reduction in NAA/Cr and Li et al. (2006) found lower NAA/Cr was correlated with lower gray matter density in the left hippocampus.

Eleven studies (2, 9, 10, 13, 14, 15, 18, 19, 20, 21 and 22) investigated NAA levels within the ACC. Several earlier studies (2, 10, 13, 14 and 15) did not specify the exact voxel location within the ACC, two of these studies (13 and 14) identified lower absolute NAA, and two studies found lower NAA/Cr (2 and 15). One study (9) reported lower NAA/Cr in the dACC when PTSD was compared to healthy controls and one other (19) reported no difference in NAA in the dACC, noting that study 19 compared PTSD to trauma exposed controls. One study in the rACC (21) identified lower absolute NAA, while the remaining three studies (19, 20 and 22) investigating this region found no significant difference in NAA. No studies have explored the longitudinal change in metabolites in the ACC with PTSD.

NAA was found to be lower in several other regions of the brain, including the left basal ganglia (Lim et al., 2003) and the parietal occipital gray matter (Meyerhoff et al., 2014). Otherwise, no significant difference in NAA was identified in the periventricular white matter (WM) (Lim et al., 2003), occipital gray matter and white matter (Seedat et al., 2005; Villarreal et al., 2002), right insula (Rosso et al., 2014), dorsolateral pre-frontal cortex (DLPFC) (Michels et al., 2014), posterior occipital cortex (POC) and temporal lobe (Meyerhoff et al., 2014; Pennington et al., 2014).

3.2 Glutamate and Glutamine

It is possible that glutamatergic dysfunction reported in several brain regions plays a role in the pathogenesis of PTSD, however findings were mixed. It is worth noting that the accurate measurement of Glu and Gln as separate entities is currently only possible at the higher field strength of 7T (Terpstra et al., 2016). Six studies (19, 20, 21, 22, 23 and 24) investigated Glx levels in multiple brain regions. In the temporal lobe two studies (19 and 21) identified a reduction in Glx and Glu respectively. Furthermore, a study performed by Pennington et al. (2014) identified higher absolute Glx in the temporal lobe in PTSD participants with co-morbid alcohol use disorder (AUD) when compared to PTSD participants without AUD. Only one study (22) identified a reduction in Glx within rACC using 1D MRS; where the authors noted that Glx was lowest in PTSD subjects followed by those in PTSD remission. Other investigators reported no difference in Glx in the ACC (studies: 19, 20, 21 and 23). More recently Rosso et al. (2017) quantified Glu and Gln in the hippocampus, using a novel TE averaging sequence 2D JPRESS; they found higher absolute Glu and Glu/NAA in the right hippocampus. Statistically significant absolute Glu concentration was positively correlated with re-experiencing symptoms and trauma dose was significantly positively correlated with right hippocampal NAA/Cr. No differences in Glu or Gln were identified in the POC or DLPFC (Meyerhoff et al., 2014; Michels et al., 2014; Pennington et al., 2014).

3.3 GABA

GABA was investigated in four studies (18, 19, 20 and 21). No difference in GABA/Cr was reported in the dACC (study 18), however, one study (20) identified higher GABA/Cr in the rACC and DLPFC. Lower GABA/Cr was noted within the insula in a single study (18) and lower absolute GABA in the POC and medial temporal lobe (study 21). One study (19) found no difference in absolute GABA in the POC when PTSD participants were compared to healthy controls, however, they did find higher GABA when PTSD sufferers with AUD were compared to PTSD participants without AUD. All the studies described here used MEGA-PRESS to quantify GABA within the brain.

3.4 Myo-Inositol

Only two studies (10 and 19) identified differences in mI in PTSD. Seedat et al. (2005) found higher mI/Cr in the ACC of participants who had PTSD secondary to intimate partner

violence. No other studies noted mI to be raised in the ACC. In a single study performed in PTSD patients with AUD, absolute mI was lower in the ACC when compared to trauma exposed controls (Pennington et al., 2014). Multiple studies (7, 13, 21 and 22) found no difference in mI in patients with PTSD, irrespective of brain region.

3.5 Choline

Twelve studies (1, 2, 7, 9, 10, 12, 13, 15, 17, 19, 21 and 22) have measured Cho containing compounds in PTSD. The majority of studies (2, 9, 13, 21 and 22) found no difference in Cho containing compounds in the ACC and hippocampus (12, 13, 15, and 17). Conflicting results were reported by two studies (10 and 15) measuring Cho in the ACC using 1D MRS Seedat et al. (2005) found higher Cho/Cr in PTSD when compared to healthy and trauma exposed controls, while Guo et al. (2012) identified lower Cho/Cr in the ACC in PTSD compared to healthy controls in a large cohort. Conflicting results were also reported in the hippocampus (studies 1 and 9). Lower absolute Cho was found in the temporal lobe of participants with PTSD and AUD when compared to PTSD participants without AUD and trauma exposed controls (Pennington et al., 2014; Seedat et al., 2005).

3.6 Glutathione

One study (20) identified an increase in glutathione/Cr in the ACC and DLPFC in PTSD when compared to trauma exposed controls.

3.7 Total Creatine

Two early studies identified a reduction in absolute Cr in the hippocampus and occipital white matter (studies: 2 and 3). Four subsequent studies have identified no difference in absolute Cr concentration (studies 13, 18, 19 and, 21).

3.8 Metabolic correlates

Increased hippocampal exposure to glucocorticoids has been proposed as a cause of atrophy in PTSD. To explore the glucocorticoid hypothesis further, two studies correlated serum cortisol levels with hippocampal neuro-metabolites and found differing results. Shu et al. (2013b) identified a negative correlation between serum cortisol and hippocampal

NAA/Cr levels, whilst Neylan et al. (2003) (not listed in Table 1 due to overlap with Schuff et al.'s (Schuff et al., 2001) population) found a positive correlation between the two.

Two studies have identified correlations with GABA and clinical outcomes in PTSD. Meyerhoff et al. (2014) noted that lower levels of GABA and higher Glu correlated with a higher insomnia severity index. In a small group of participants, Rosso et al. (2014) found lower levels of GABA were correlated with higher state-trait anxiety levels. However, they found no correlation between GABA and severity of symptoms (Rosso et al., 2014).

Other reports identified a correlation with re-experiencing symptoms and NAA levels. Ham et al. (2017) identified lower levels of NAA in the ACC were correlated with re-experiencing symptoms and more recently, Rosso et al. (2017) found the same in the hippocampi of PTSD subjects. Shu et al. (2013b) noted a correlation between lower NAA/Cr levels in the hippocampus and higher total clinical administered PTSD scores (CAPs). Otherwise, no significant correlation between NAA and symptom severity was identified (Kimbrell et al., 2005; Lim et al., 2003; Rosso et al., 2017).

A positive correlation between absolute Glu levels in the right hippocampus and re-experiencing symptoms was identified by Rosso et al (Rosso et al., 2017). Additionally, Harnett et al. (Harnett et al., 2017) found a positive relationship between dACC and absolute Glx levels and current and future stress disorder symptoms.

A positive correlation was found between disease duration and the anti-oxidant glutathione levels (Michels et al., 2014).

3.9 Confounding variables in PTSD research

Current or past alcohol dependence is a common co-morbidity seen in PTSD that has been shown to result in significant differences when PTSD participants with and without AUD are compared (Pennington et al., 2014) and this is likely to be particularly important for GABA, NAA and Glx (Behar et al., 1999).

Multiple studies have controlled or adjusted for the effects of depression and medications (mainly serotonin re-uptake inhibitors) using a linear regression and have found no significant effect (Eckart et al., 2012; Kimbrell et al., 2005; Lim et al., 2003; Michels et al., 2014). In addition, no significant reduction in NAA has been reported in the medial temporal lobe / hippocampus or hippocampi in depression (Rao et al., 2011).

Many studies have specifically excluded participants with traumatic brain injury (TBI), given that these individuals may have a higher risk of PTSD, which can be related to the TBI or to a separate event (Bryant, 2001; Kennedy et al., 2007; King, 2008). None of the studies identified directly compared TBI and PTSD groups.

Two studies compared PTSD subjects to traumatised and non-traumatised control groups (Eckart et al., 2012; Seedat et al., 2005), in an effort to determine if an observed effect was due to PTSD or simply trauma exposure. These studies have had mixed results; Seedat et al. (2005) found a significant difference between PTSD and trauma exposed control groups, in the ACC, suggesting that the observed metabolic difference was due to PTSD itself. On the other hand, Eckart et al. (2012) found no significant difference between the PTSD, healthy / trauma exposed control groups using bilateral hippocampal and insula voxels.

4 Discussion

The current systematic review has summarised findings from 24 proton MRS studies comparing PTSD subjects to controls subjects. There is emerging evidence of neurochemical abnormalities in patients with PTSD, identified using in vivo proton MRS of the human brain. It is important to note that the hardware and post-processing capabilities for in vivo neuro MRS have been steadily improving as early studies were restricted in what they were able to record.

The most consistent findings to date are reductions in NAA in the region of the hippocampus and ACC. Multiple studies have found reductions in both absolute NAA and NAA/Cr in both hippocampi in PTSD participants when compared to healthy and trauma exposed controls. A meta-analysis of MRS studies performed by Karl et al. (2010) identified lower NAA in the left hippocampus when compared to healthy and trauma exposed controls

and lower right hippocampal NAA only when compared to trauma exposed controls. Two possible aetiologies are explained for the laterality of lower hippocampal NAA: the left hippocampus has a larger volume in right handed individuals and perhaps atrophies faster and the left hippocampus has a greater role in declarative memory (encoding and retrieving tasks) and declarative memory is known to be impaired in PTSD (Li et al., 2006). Since publication of Karl and Werner's meta-analysis (Karl and Werner, 2010), there have been three additional studies published that have identified lower NAA/Cr (Guo et al., 2012; Shu et al., 2013) and lower total NAA (Rosso et al., 2017) in both hippocampi of PTSD participants. NAA can be reduced in the brain for multiple reasons (see '*1.2 Important Brain Metabolites*' above), one of which is lower neuronal density, ultimately leading to morphologic change or atrophy. The findings of lower NAA within both hippocampi correlates with the meta-analyses of structural imaging studies (Kitayama et al., 2005; Smith, 2005). It is still not clear from the data published to date if the MRS differences (lower NAA) precedes morphological changes, however, this would appear very likely, given that multiple studies have identified lower hippocampal NAA without atrophic change. It is worth noting that a large number of studies identified no difference in hippocampal NAA despite the fact that almost all of these studies were performed on veterans and participants with long standing chronic PTSD. This may suggest that the reduction in hippocampal NAA is reversible in PTSD, as has been shown in conditions such as acute brain injury or methamphetamine abuse (Maddock and Buonocore, 2012), which is thought to represent reversible neuronal or mitochondrial dysfunction (Moffett et al., 2007) as described in the introduction. Additionally, the majority of the studies that found lower NAA in the hippocampi of those with PTSD compared to healthy controls, suggesting that the difference is not due to the choice of control cohort (trauma exposed vs. healthy control), as proposed by Karl et al. (Karl et al., 2006).

A smaller number of studies have reported a reduction in NAA within the ACC with increased heterogeneity of results when compared to the hippocampus. However, many of the early studies did not specify the region of the ACC being measured. Studies that have reported NAA in the rACC have given mixed results, some with lower NAA and others with no difference. Differences in the rACC in PTSD are thought to be acquired and the disparity

in the MRS results may be due variations in metabolite concentrations over time (Admon et al., 2013).

More recently, with advancing spectroscopic techniques, researchers have identified glutamatergic dysfunction and differences to GABA in some of the brain regions where we would expect hypoactivation, based on previous fMRI work, such as the occipital cortex and rACC. In a study of adolescents exposed to earthquake, Yang et al. (Yang et al., 2015) found lower Glx/Cr in the rACC, in both PTSD and remitted PTSD. However, patients with PTSD had further lower rACC Glx/Cr when compared to remitted PTSD patients, suggesting lower rACC excitatory neurotransmission and therefore lower amygdala inhibition. However, this finding was not supported by Meyerhoff et al. (Meyerhoff et al., 2014), which may be due the fluctuating of metabolic differences over time in this region. On the other hand, higher Glx has been identified in the rACC of patients with PTSD and comorbid AUD when compared to PTSD patients without AUD (Pennington et al., 2014) and with an associated increase in GABA in the same region (Meyerhoff et al., 2014), raising the possibility that alcohol reverses some of the differences described above and partly explains the association of PSTD and AUD. Higher GABA/Cr has been noted in the rACC.

One hypothesis for higher GABA is overexpression of Glu or Gln decarboxylase and/or lower clearance of GABA from the synaptic cleft (Michels et al., 2014), which supports the hypothesis of rACC hypoactivation, resulting in amygdala hyperactivation (Admon et al., 2013). More recently, higher absolute Glu was identified in the right hippocampus (Rosso et al., 2017) raising the possibility that reductions in volume and NAA within the hippocampus may be secondary to excitotoxicity.

No difference in GABA or Glx have been identified in the dACC, noting that only a small number of studies have evaluated this region. fMRI studies have shown this region to be hyperactive in PTSD and the region may be congenitally abnormal in those who have a risk of developing PTSD. Future studies aiming to predict those at risk of developing PTSD may be able to further explore this region.

A small number of studies have reported altered Cho levels in PTSD, but the results are inconsistent. The two studies that identified differences in Cho used relative measures and compared PTSD to healthy controls with conflicting results. One study found a reduction in

absolute Cho in the ACC in a population with comorbid AUD (Pennington et al., 2014). Alterations in Cho may be due to differences relating to glial cells (Seedat et al., 2005), white-matter or could reflect neuroinflammation (Rohleder and Karl, 2006). More work is needed to determine the directionality of differences in Cho containing compounds.

Only one study investigated metabolic differences in the insula cortex, finding lower GABA/Cr with a significant negative correlation with state and trait anxiety. This finding correlates with previous fMRI studies that have identified increased insula cortex activation in PTSD, social anxiety and specific phobia disorders (Etkin and Wager, 2007).

Increasing glutathione correlated with disease duration (Michels et al., 2014). Glutathione is an antioxidant that reduces reactive oxygen species, which can have a detrimental effect at a cellular level. It is not yet known if higher glutathione is a predisposing factor or acquired as the result of PTSD, however, mouse models have also shown overexpression of the genes glyoxalase 1 and glutathione reductase 1 results in higher anxiety, linking the glutathione cycle with anxiety behaviour in a mouse model (Hovatta et al., 2005).

4.1 Limitations

A challenge for all neuroimaging studies exploring PTSD is the heterogeneity of patient populations. PTSD is known to have multiple comorbidities such as Axis I and II disorders, including depression; alcohol and substance misuse and chronic pain (Scioli-Salter et al., 2015). Additionally, the majority of patients with PTSD are taking medications, a factor that may confound interpretation of neurochemical differences (Lanius, 2010). These factors can make generalising the study results and determining unique metabolic differences in PTSD problematic. There is evidence that AUD alters brain metabolites (Schuff et al., 2008) and these participants with AUD should be excluded. Depression has not been shown to contribute to metabolic differences recorded in PTSD and co-morbid depression is so common in this group one could argue that excluding those with depression would result in an unrepresentative sample. Medications such as benzodiazepines and antipsychotics are highly likely to alter neuro-metabolites and should be controlled for. It is less clear if serotonin re-uptake inhibitors are used, but to date no significant difference has been

identified in participants using these drugs (as discussed in section 3.9). A limited number of studies have identified differences between PTSD, healthy control and trauma exposed groups and to ensure that the differences being identified can be attributed to PTSD alone most studies use a trauma exposed control group.

Many of the early spectroscopic studies (55%) utilised a low field strength (1.5T), to measure metabolic differences in patients diagnosed with PTSD. There are inherent limitations in performing neurospectroscopy at low field, such as increased peak overlap; limiting the number of measurable metabolites. Additionally, many of the early studies used peak area or peak fitting with a limited basis set to quantify a limited number of metabolites, such as NAA, Cr and Cho, a technique that has now been superseded by peak fitting with more extensive basis sets and bioinformatics evaluation of the data point by point (Stanwell et al., 2010).

Several early studies used a larger MRS voxel within the medial temporal lobe that included the hippocampus. The benefit of this approach is that signal to noise ratio is higher, the limitation is the spectrum contains contributions from surrounding medial temporal lobe parenchyma. As MRI hardware and techniques have improved, researchers have been able to reduce the MRS voxel size, whilst maintaining adequate signal-to-noise and improving the anatomical specificity of the MRS voxel. This difference in voxel volume and anatomic location, may also go some of the way to explaining the variability in MRS findings in the hippocampus.

Finally, there is a large degree of methodological heterogeneity within studies due to the differing MRS pulse sequences available, such as PRESS and STEAM for 1D and the availability of 2D magnetic resonance spectral imaging (MRSI). To further complicate comparisons there are multiple ways in which data can be post-processed, evaluated and corrected. For example, fitted 1D MRS metabolite peaks can be referenced to an internal resonance such as Cr or referenced to tissue water concentration, known as absolute quantification. Some perform partial volume corrections. However, the spectroscopy community have recognised this limitation and recommended protocols for common use (Oz et al., 2014).

5 Conclusions and Future directions

The most consistent MRS findings in PTSD is lower NAA in the hippocampi and ACC. The reduced NAA in these regions may be due to neuronal loss and early evidence suggests that reductions in NAA may proceed morphologic change. On the other hand, multiple studies have demonstrated no difference in NAA, raising the possibility that reductions in NAA in PTSD may be reversible or fluctuate over time. Levels of NAA in the ACC and hippocampus have been found to correlate with clinical symptoms by some. There is emerging evidence of glutamatergic dysfunction in the hippocampus, occipital cortex and rACC and it is possible that higher levels of Glu in the hippocampus may be contributing to neuronal loss, secondary to excitotoxicity. Mixed differences have been found in GABA, which is not unexpected due to the dynamic nature of this metabolite. The majority of studies have found no change in mI or Cr, however caution is recommended when using Cr as an internal reference. Further work is required to determine the magnitude and direction of change in Cho containing compounds in PTSD, which to date appear to be reduced or unchanged. A single study has measured glutathione, suggesting an increase in PTSD. MRS studies have been able to identify metabolic changes in the brains of patients with PTSD, a condition that has no conventional imaging findings. Ongoing work is required to further characterise these differences, whilst attempting to maximising the generalisability of results.

Many of the early spectroscopic studies were limited by hardware. In the last decade, there have been multiple technological leaps in both MR scanner technology, shimming and acquisition protocols (Oz et al., 2014). Higher magnetic field strengths allow for higher signal to noise and greater peak separation, improving comparative accuracy. The newer hardware has made techniques such as 2D and 3D MRSI, highly relevant to the study of PTSD. High spatial resolution 3D MRSI, performed at high field strengths (3 and 7T), will allow for measurement of metabolites in brain regions such as the amygdala (a region that it yet to be examined due to its small volume), insular cortex and the hippocampus. Additionally, k-space under sampling can be utilised to accelerate the acquisition of 3D MRSI, lowering acquisition times. Two-dimensional MR spectroscopy is a technique that allows overlapping and composite metabolic peaks to be deconvoluted (Ramadan et al.,

2011; Thomas et al., 2003); it has been now applied to several pathologies and offers further metabolic insights not available using 1D MRS alone (Lin et al., 2015; Ramadan et al., 2011; Ramadan et al., 2015). However, it is yet to be applied to the condition of PTSD and may allow identification of novel biomarkers that have not been identified using 1D MRS.

Another promising technique, which is yet to be applied to PTSD is functional MRS (fMRS). fMRS is a technique that characterises the regional and temporal change of neurotransmitters whilst subjects are exposed to a stimulus, as in fMRI which measures differences in blood oxygen level. This technique is highly relevant to PTSD and may yield further insights into the pathogenesis of the condition. For example, fMRS could be applied to the hippocampus, using a learning task, in participants with PTSD to further evaluate differences in glutamatergic modulation within the hippocampus. Finally, fMRS may improve MRS reproducibility, by controlling for behaviour, potentially reducing the variability of studies quantifying neurotransmitters.

Machine learning techniques are providing a more sophisticated and tailored means of evaluation of the complex, but rich data available from in vivo 1D and 2D neuro MRS, as well as the evaluation of multimodal MRI data. Machine learning techniques may improve the specificity and sensitivity for evaluation of the PTSD condition when using MRS. Machine learning techniques have been used in the past for the analysis of 1D MRS evaluating chronic pain and primary brain lesions (Ranjith et al., 2015; Stanwell P et al., 2010), increasing the specificity of MRS for detecting these conditions, but as yet have not been applied to PTSD.

There is a need for further prospective studies in the field of PTSD using advanced neuro MRS, techniques. These studies should aim to answer the following questions: does lower hippocampal volume predispose to PTSD; does metabolic differences in the hippocampus proceed atrophy?; are there MRS differences in the dACC that predispose to PTSD and are rACC and insular cortex MRS differences acquired as part of the condition?.

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Tables 1.

Study	Voxel Location/ Size	Field / Sequence / TR (s) / TE (ms) / averages	Participants	Exclusion Criteria	Trauma	Psychological Assessment	Mean CAPS	Findings	Comments
1 (Freeman et al., 1998)	Hippocampi / 20 x 20 x 30 mm^3	1.5T / STEAM / 2 / 30 / 128	21 PTSD subjects 8 veteran controls.	Head injury Dementia Cognitive impairment.	Combat related trauma	CAPS SCID	NG	Right hippocampus: ↓ NAA/Cr ratio Left hippocampus: ↓ Cho/Cr	Did not control for alcohol, substance dependence or handedness. Comorbid major depression. Matched for age and education. No PV correction. SPARC workstation for calculation of peak ratios.
2 (De Bellis et al., 2000)	ACC / 20 x 15 x 10 mm^3	1.5 / STEAM / 1.5 / NG / 150	Children and Adolescents 11 PTSD subjects. 11 Healthy controls. Matched for age, sex and IQ. 2 subjects left handed.	Lifetime exposure to psychotropic drugs A significant medical or neurological illness or history of head injury Gross obesity or growth failure Full scale IQ lower than 80 Presence of DSM-IV anorexia nervosa, autism, substance use disorder, or schizophrenia History of maltreatment of axis I disorder in controls.	Sexual abuse Physical abuse Witnessing domestic violence.	K-SADS-PL Wechsler Intelligence Scale for Children.	NG	ACC: ↓ NAA/Cr ratio. No difference in Cho/Cr.	NAA/Cr calculated with LC-Model.
3 (Schuff et al., 2001)	Hippocampi / 210 x 210 x 15 mm^3	1.5T /PRESS CSI / 1.8 / 135 / NG	18 male PTSD. 19 male HC.	Alcohol dependence in the last 5 years Illicit drugs prior 5 years LOC after head trauma Major depression last 3 months Antipsychotics last 6 months.	Combat Veterans	SCID (DSM-IV) CAPS	63	Hippocampi: ↓NAA and Cr in both. Absence of hippocampal atrophy.	Absolute (arbitrary units). Quantified using in house software. Partial volume corrected Controlled for alcohol dependence (5yrs) prior) and found no hippocampal atrophy.
4 (Mohanakrishnan Menon et al., 2003)	Hippocampi / 15 x 15 x 15 mm^3	1.5T / PRESS / 3 / 30 / 128	14 PTSD. 7 controls (Veterans).	Seizure disorder > 100mg/day Gabapentin Severe head injury with LOC Uncontrolled diabetes Chronic hypertension Chronic alcohol or substance abuse Stroke.	Vietnam veterans. Two participants with non-combat trauma.	Diagnosis made according to the DSM IV. PCL	CAPS not recorded.	Left Hippocampus: ↓ NAA/Cr ratio Right hippocampus: no sig difference in NAA/Cr. No clinical correlations performed.	tCr used as the internal ref. Hippocampal volume was not calculated. Not controlled for handedness, medications (other than gabapentin), recent alcohol or substance abuse. No regression.
5 (Villarreal et al., 2002)	Hippocampi / 15.3 x 20.3 x 30 mm^3 Bilateral occipital WM / 20 x 20 x 21 mm^3	1.5T / PRESS / 2 / 40 / 128 1.5T / STEAM / 2 / 30 / 128	8 PTSD. 5 Healthy controls.	Major medical or psychiatric diagnosis Alcohol or substance dependence History of head trauma with LOC Seizures Neurological Disorder.	Mixed. Including child sexual assault	SCID-P (DSM-IV) CAPS Beck Depression Inventory Beck Anxiety Inventory.	>60	Left hippocampus: Trend to ↓ NAA (p =0.054). Occipital WM: ↓ Cr no difference in NAA.	Absolute (arbitrary units). Quantified using in house software. Hippocampal volume not quantified. No clinical correlates performed. Comorbid depression with medication use in some participants, including benzodiazepines. Matched for handedness.

6 (Brown et al., 2003)	Right and left medial temporal lobes / 20 x 20 x 20 mm^3	1.5T / STEAM / 2 /30 / 128	9 POW + PTSD. 12 POW – PTSD.	No history of TBI with LOC No neurological impairment Degenerative neurological condition Dementia. No participants met criteria for current or lifetime alcohol dependence.	Former prisoners of war, with combat experience.	CAPS-2 SCID (DSM-IV)	72	No significant difference between groups. Left MTL: Trend toward ↓ NAA/Cr. Negative correlation between NAA/Cr in the MTL bilaterally and CAPS-2 re-experiencing symptoms.	tCr used as internal reference. Controlled for alcohol, handedness, alcohol dependence. Brain volumes not quantified.
7 (Lim et al., 2003)	Left basal ganglia, right frontal periventricular WM and right parietal periventricular WM / 20 x 20 x 20 mm^3	1.5T / STEAM / 3 / 30 / 36 / (mixing time 13.7ms)	16 PTSD (10M:6F). 8 Healthy controls.	Organic mental, psychotic, bipolar, psychotic or neurological disorders. Major head injury (LOC >10min) Alcohol or other substance dependence in the last year No psychotropic medications for the previous 4 weeks.	Fire - Public cafe in Korea 1999	SCID -RV	61	Left basal ganglia: ↓ NAA/Cr FWM: No sig. diff. NAA/Cr or Cho/Cr PWM: No sig. diff. NAA/Cr or Cho/Cr No correlation with symptom severity.	Homogenous population. No partial volume correction. tCr used as the internal ref. Controlled for alcohol and medications.
8 (Kimbrell et al., 2005)	Medial temporal lobes / 20 x 20 x 30 mm^3	1.5T/ STEAM / 2/ 30/ 128 / mixing time 13.7ms)	47 PTSD. 21 Veteran controls (no previous combat exposure).	No head injury with LOC Left handed No prior neurological illness Controls screened for psychological illness using SCID.	War related trauma Subdivided into combat and non-combat groups.	SCID (DSM-IV) CAPS WSM III WASI MAST DAST BDI SCL-90R	NG	Left MTL: NAA/Cr higher in PTSD-C vs PTSD-NC. Right MTL: no difference. No difference between NAA/Cr in the MTL between HC and PTSD. No correlation with NAA/Cr in either MTL and severity of PTSD.	Did not consider other traumas. Cr reference. No partial volume correction. Did not consider childhood abuse. Significant difference between the age of subjects and controls (44.5 vs 48.4). Controlled for handedness and prior mTBI. Matched for depression.
9 (Mahmutyazicioglu et al., 2005)	Hippocampi / 30 x 11 x 12 mm^3 dACC / 30 x 20 x 10 mm^3 or 30 x 15 x 16 mm^3	1.5T / PRESS / 2 / 136 / 128	10 PTSD 6 Healthy controls	Alcohol or substance abuse during the last 6 months. Use of drugs of any kind Claustrophobia Pregnancy MR incompatible metal prosthesis / implant.	Antiterrorist combat Witnessing the death of a friend in a fire Childhood sexual abuse Trapped in a landslide.	CAPS	64	Hippocampi: ↓ NAA/Cr and ↑ Cho /Cr dACC: ↓ NAA/Cr and no difference in Cho /Cr.	Visual scoring (Scheltens et al) for hippocampal atrophy. No atrophy reported. Cr reference. Peaks calculated on the Phillips scanner. No partial volume correction. Small groups.
10 (Seedat et al., 2005)	ACC / 10 x 10 x 30 mm^3 Left occipital gray matter / 10 x 10 x 30 mm^3.	1.5T / PRESS / 1.5 / 135 / 256	7 female PTSD. 9 female trauma exposed controls. 11 non-trauma exposed controls.	Psychotic disorder Bipolar disorder Psychotropic medications within 6 weeks Substance use disorder within 1 year > 2 years of alcohol abuse Neurologic disorder or head injury associated with cognitive dysfunction History of seizure disorder ADD or learning disability Pregnancy or HIV.	Intimate partner violence - out of relationship for 4 months but not >2yrs.	SCID-P (DSM-IV) CTS -2 Stroop	CAPS not recorded.	ACC: ↑ Cho /Cr and ↑ mI/Cr in PTSD subjects when compared to TEC. ACC: no significant difference when PTSD/TEC's were compared to HC. Occipital GM: No difference in metabolites. No correlation with Stroop test or the severity of IPV.	tCr used as the internal ref.
11 (Li et al., 2006)	Hippocampi / 15.3 x 20.3 x 40 mm^3	1.5T / PRESS / 1 / 144 / 248	Prospective case-control study. 12 PTSD. Recent Diagnosis. 12 Trauma exposed controls.	No alcohol or other substance use disorder within 1 yr. No psychotropic medications.	Fire in Hunan Province China. All participants exposed to the trauma.	Psychiatrist interview. Diagnosed according to SCID DSM-IV. DEQ	CAPS not recorded.	Left hippocampus: ↓ NAA/Cr. Using VBM identified reduced gray matter density of the left hippocampus.	Didn't measure handedness. tCr and Cho metabolite ratios calculated. Small number of participants with major depression. No

									medications.
12 (Freeman et al., 2006)	Hippocampi / 10 x 10 x 40 mm^3	1.5T / PRESS / 2 / 144 / 128	POW + PTSD POW – PTSD Control	Female sex Left handed History of TBI with LOC No history of neurological impairment or degenerative neurological illness Current or lifetime alcohol dependence MMSE >26.	Prisoners of war	CAPS-25 SCID Edinburgh handedness inventory Beck depression inventory Davidson trauma scale Combat exposure scale Rey Auditory Verbal Learning Test, Logical Memory Recognition Memory Test for Faces.	POW + PTSD: 53 POW – PTSD: 14 Control: 4	Hippocampi: No statistical difference in NAA/Cr or Cho/Cr in either group. No difference in hippocampal volumes.	Controlled for gender, age and education. Processed using SPARC workstation. Hippocampal volume manual determined.
13 (Ham et al., 2007)	ACC / 15 x 15 x 15 mm^3 Hippocampi / 15 x 15 x15 mm^3	3.0T / PRESS / 2 / 35 / 128	26 PTSD subjects. 25 age and gender matched HC.	Current or past significant medical illness Physical injury from fire >10% BSA burn LOC during escape Any axis I psychiatric diagnosis Antisocial or borderline personality disorder Lifetime exposure to any substance other than nicotine, moderate alcohol use and caffeine. ADHD IQ < 80 Contraindication to MRI scanning.	Fire in subway train Taegu, South Korea. February 2003.	SCID (DSM IV) CAPS Hamilton Depression rating scale Hamilton anxiety rating scale.	71	Hippocampi: ↓NAA ACC: ↓ NAA. No difference in Cr, Cho or mI. NAA in the ACC negatively correlated with re-experiencing symptoms. Did not quantify hippocampal volume.	Absolute quantification performed with unsuppressed H_20.
14 (Schuff et al., 2008)	Hippocampi / 7.8 x 7.8 x 15 mm^3 Frontal and parietal lobes / 8.5 x 8.5 x 15 mm^3 (two slices)	1.5T / PRESS HMRSI / 1.8/ 35 / NG	PTSD with and without alcohol abuse (28 /27). Trauma exposed controls with and without alcohol abuse (23 /26).	Past PTSD Psychotic disorder Bipolar disorder Drug abuse or dependence (last 6 months). Neurological illness Head trauma with LOC Medical disorder affecting brain function MRI exclusion criteria Brain tumour, small vessel disease, WM lesions on MRI.	Combat Military Service Civilian events Childhood abuse	CAPS SCID (DSM IV) LSC-R Total cumulative alcohol over 5 years.	CAPS +A group: 66 CAPS -A group: 63	Hippocampus: ↓NAA/Cr without smaller hippocampal volume (either group.). No significant reduction in absolute NAA. ACC: ↓NAA (absolute) Alcohol had no significant effect on the brain volumes. Trend toward smaller hippocampal volume in subjects with prior childhood trauma. Depression has no effect on the regression model.	Large groups. Noting that data for 19 subjects was not of good quality. A large number of participants in both groups had previous childhood abuse. Absolute (arbitrary units) concentration determined according to (Schuff et al., 2001). Metabolite ratios also calculated, normalised to Cr. ACC region not further specified.
15 (Guo et al., 2012)	Hippocampi / 10 x 10 x 10 mm^3 ACC / 10 x 10 x 10 mm^3	1.5T / PRESS / 1 / 144 / NG	50 PTSD. 50 heathy controls. Age and gender matched groups.	Antipsychotics, antimanic, antidepressant or benzodiazepine drugs. History of LOC > 5 minutes Clear diagnosis of neurological disease Serious body disease Clear diagnosis other mental disorder History of alcohol or morphine abuse History of childhood abuse.	PCL score >= 44 CAPS > 60 <1-year duration of PTSD	PCL-C CAPS WAIS	CAP not reported	ACC: ↓NAA/Cr and ↓Cho/Cr. Hippocampi: ↓NAA/Cr.	ACC location not further specified. Data analysis performed using Functool 2 and ACD 4.0. tCr used as the internal ref. Volume quantification not performed.
16 (Eckart et al., 2012)	Hippocampi (medial and posterior portion) / 20 x 10 x 10 mm^3 Bilateral insula / 30 x 10 x 15 mm^3	3.0T / PRESS / 2 / 135 / 256	20 PTSD. 16 Trauma exposed controls. 11 Healthy controls. All male.	Lifetime or current abuse of substances (particularly alcohol) Neurologic diseases Any contraindication for magnetic resonance imaging (MRI) Psychiatric conditions other than PTSD or major depression.	Highly traumatised refugees. Trauma exposure occurred at mean age of 15.	CTQ Vivo checklist of War, Detention and Torture Events CAPS MINI MP test	CAPS PTSD: 69 CAPS: TEC: 14	No significant difference in the absolute NAA or NAA/Cr between PTSD and HC in any brain region. No significant difference in the absolute NAA or NAA/Cr between PTSD and TEC in any brain region. No difference in the hippocampi or the insula.	Absolute quantification performed with unsuppressed H_20. LC Model. Partial volume correction performed.

									Volume quantification of hippocampi and insula. Only one PTSD subject was taking psychoactive medications. Co-morbid major depression (n=15 PTSD). Inclusion of depression as a co-variate did not alter results.
17 (Shu et al., 2013)	Hippocampi / 7.5 x 7.5 x 10 mm^3	1.5T / PRESS-CSI / 1/ 144 / 2	11 PTSD (right handed). 11 age, gender and education matched HC.	Bipolar disorder Schizophrenia or psychotic disorder Alcohol or Substance abuse or dependence in past 5years Physical or sexual abuse during childhood Current or past history of neurological disease Other physical diseases identified by EEG or MRI.	Mixed: including sexual assault.	CAPS	Mean CAPS 84.9	Hippocampi: ↓ NAA/Cr No difference in Cho. Significant reduction in left and total normalised hippocampal volume when compared to controls. Volume of the left hippocampus was negatively correlated with CAPS total and CPAS-C scores. Serum cortisol negatively correlated with right hippocampal NAA/Cr ratio.	PTSD group receiving anti-depressants and anti-psychotics. tCr used as the internal ref. GE ADW Functool used to quantify peaks area. Controlled for handedness, age, gender and education.
18 (Rosso et al., 2014)	Right Insula / 15 x 30 x 20 mm^3 dACC / 30 x 20 x 20 mm^3	4.0T / MEGA-PRESS / 2 / 68 / 384	13 PTSD. 13 Healthy controls.	Medical condition: mTBI, LOC, seizures Current substance use disorder Nicotine use Use of benzodiazepines or anxiolytic anticonvulsant Mood stabiliser or neuroleptic medication within 4 weeks of the study Substance abuse in the last five years Structural abnormality on MRI scan Recent psychoactive drug (urine screen). Pregnancy.	Assault Childhood physical abuse Childhood sexual abuse Combat exposure Rape Multiple traumas	SCID - I CAPS STAI-S/T BDI	62	Insula: ↓ GABA/Cr. No association with symptom severity. Negative correlation with state and trait anxiety. dACC: GABA/Cr: no difference between groups. No difference in Cr or NAA.	Voxel located within the dACC. Spectrum pre-processed and then fitted using LC Model. tCr used as the internal ref. Matched for menstrual cycle. Almost all patients free of psychotropic medications. Controlled for drugs, nicotine and alcohol.
19 (Pennington et al., 2014)	rACC / 35 x 25 x 20 mm^3 POC / 40 x 20 x 20 mm^3 TEMP / 20 x 40 x 20 mm^3	4.0T / MEGA-PRESS / 2 / 68 / 384 4.0T / STEAM / NG / 16 / NG	10 PTSD with AUD. 28 PTSD without AUD. 20 Trauma exposed controls.	Schizophrenia or schizoaffective disorder Past or current alcohol dependence (control) Suicidal intention Bipolar Suicidal ideation Seizure disorder Head injury with (post injury mem loss >24hrs or LOC 10min) Stroke Neurodegenerative condition HIV Medically unstable Psychotropic medication last two weeks.		CAPS ISI AUDIT Time line follow back Beck Depression Inventory - II Beck anxiety inventory.	PAUD: 78.6 PTSD: 55.2 CON: 2.7	TEMP: lower NAA in PAUD than PTSD and TEC. No diff PTSD vs TEC. TEMP and ACC: ↓ Cho in PAUD compared to TEC and PTSD. rACC: ↓ mI in PAUD in ACC when compared to TEC and PTSD. TEMP: ↑ Glx in PAUD vs PTSD. No difference PAUD vs. TEC. POC: ↑ GABA in PAUD vs PTSD. No difference PAUD vs. TEC. Within PAUD, higher GABA and Glu correlated with improved cognition.	Absolute concentrations (IU). ACC voxel contained primarily within the rostral ACC.
20 (Michels et al., 2014)	DLPFC / 25 x 40 x 30 mm^3 rACC / 28 x 30 x 22.5 mm^3	3.0T / MEGA-PRESS / 1.8 / 68 / (320 DLPFC and 640 ACC)	12 PTSD (Some on medication). 17 Trauma exposed controls.	Free of neurological and other major medical conditions.	Mixed	Trauma History - PDS Childhood trauma questionnaire SCID-I SCID-I-R CAPS Multidimensional inventory of dissociation State trait anxiety	67	DLPFC and rACC: ↑ GABA/Cr and ↑ glutathione/Cr. No difference in NAA/Cr or Glx/Cr. Positive correlation with glutathione and disease duration.	Voxel located primarily within the rostral ACC. tCr used as the internal ref. Fitted using LC model.

						inventory Beck depression inventory Cognitive testing			
21 (Meyerhoff et al., 2014)	rACC / 35 x 25 x 20 mm^3 POC / 40 x 20 x 20 mm^3 TEMP / 40 x 40 x 20 mm^3	4.0T / STEAM / 1.8 / 15 / 128 4.0T / MEGA-PRESS / 2 / 71 / 256	Adults - Military and civilian population. 27 PTSD. 18 Trauma exposed controls.	Schizophrenia or schizoaffective disorder Alcohol dependence within 6 months Bipolar Suicidal ideation Seizure disorder Head injury with (post injury symptoms or LOC 10min) Stroke Neurodegenerative condition Medically unstable injuries related to trauma.	Combat (n=17) Non-combat (n=10)	CAPS PCL Insomnia severity index (ISI) AUDIT FTND BDI BAI STAI SCL-GSI	PTSD: 54.5	rACC: ↓NAA, no difference in Glu and GABA. TEMP: ↓GABA and ↑ Glu. POC: ↓ GABA and NAA and Glu no difference. No difference in Cr, Cho or mI in any of the groups. In PTSD POC GABA was negatively correlated with ISI (r= -0.55, p=0.008).	Absolute concentration (IU). Quantified with 'SI tools' Changed the number of averages depending in SNR (192 vs 256)
22 (Yang et al., 2015)	rACC / 20 x 20 x 20 mm^3	3.0T / PRESS / 2 / 30 / 128	Adolescents. 23 Remitted PTSD. 10 Healthy controls. 10 PTSD.	Smoking Alcohol Excess Other axis I disorder (except mood / anxiety) IQ<80 Psychotropic medication last 4 weeks Any sig. medical or neurologic condition.	Earthquake	IQ - Wechsler Intelligence Scale for Chinese Children CAPS-CA C-WISC	PTSD: 68.7 Remitted: 5.6	rACC: Lower Glx/Cr between PTSD group and HC. Glx lowest in PTSD followed by patients in remission. No difference in NAA/Cr, Cho/Cr, Glx/Cr was weakly correlated (r=-0.355, p=0.314) with CAPS-CA.	Peaks normalised to Cr. Fitted using AMARES.
23 (Harnett et al., 2017)	dACC / 20 x 27 x 10 mm^3	3T / PRESS / 2 / 30 / 128	19 Trauma exposed individuals (physical injury requiring visit to trauma unit / emergency and exposure to traumatic event on the PDS) 18 Healthy controls	History of blood or circulation disorders Diabetes Brain or spinal abnormalities Pregnancy Previous or current head injury Previous / current diagnosis of mental illness prior to event.	Physical injury requiring visit to trauma unit / emergency and exposure to traumatic event on the PDS.	PDS (at T_0 and T_1) PRFS WTAR	PDS (1 month): 13 PDS (3 month): 12.15	No difference between the groups. Glx Positive linear relationship and current stress disorder symptoms in trauma exposed participants. Glx positive linear relationship with future stress disorder symptoms.	Significant difference in participant age (4.5 years) Absolute concentration. Metabolites normalised to water. Partial volume correction performed.
24 (Rosso et al., 2017)	Hippocampi / 15 x 20 x 30 mm^3	4.0T / 2D JPRESS / 2 / 30 – 330ms / 16 (4 scans per TE)	24 PTSD. 23 Trauma exposed controls. Age and gender matched. Female participants matched for menstrual cycle.	Medical conditions affecting brain structure Current substance use disorder Current nicotine dependence Anxiolytic, anticonvulsant, mood stabilizing or neuroleptic medication use within 4 weeks of study History of substance abuse within the past 5 years Lifetime history of substance dependence Lifetime history of psychosis MR contraindications Urine test positive for psychoactive drugs or beta-HCG.	Childhood abuse (n= 5) Childhood sexual abuse (n=4) MVA or violent accident (n=2) Victim of physical assault (n =7) Combat exposure (n=1) Victims of sexual assault (n=9).	SCID / IP CAPS Traumatic life events questionnaire.	PTSD: 59.5	Hippocampi: ↓ NAA/H_20 Right hippocampus: ↑ Glu/H_20 and Glu/NAA Re-experiencing symptoms were negatively correlated with all NAA ratios. Significant positive correlation between re-experiencing symptoms and the right hippocampus Glu / H2O not for Glu/Cr. Trauma load was significantly positively correlated with right Glu /NAA in PTSD. No significant difference in the gray matter volume.	FWHM 8-12 Hz Metabolites were normalised to both water, Cr and NAA.

Table 1 - A summary of the methods and results of the MRS studies included in this review. Abbreviations: 2D-JPRESS – two-dimensional J-resolved spectroscopy; ADHD – attention deficit hyperactivity disorder; AUD: Alcohol use disorder; beta-HCG – Human chorionic gonadotropin (pregnancy marker); BSA – body surface area; DLPFC – Dorsolateral prefrontal cortex; EEG – electroencephalogram; FWM – Frontal white matter; HC – healthy control; IQ – intelligence quotient; K-SADS-PL - Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version; LOC – loss of consciousness; mTBI – mild traumatic brain injury; NA – not applicable; NG – not given; PAUD – PTSD with alcohol use disorder; PCL – PTSD check list; PDS – post traumatic diagnostic scale; POC – Posterior occipital cortex; POW – Prisoner of war; PRESS – Point-RESolved Spectroscopy; PRFS – Psychosocial risk factor

survey; PWM – Parietal white matter; STEAM – Stimulated Echo Acquisition Mode; TEC – trauma exposed control; TEMP – lateral temporal cortex; WM – white matter; WTAR – Weschler test of adult reading;. Please go to the individual manuscripts for further details of psychological assessment.

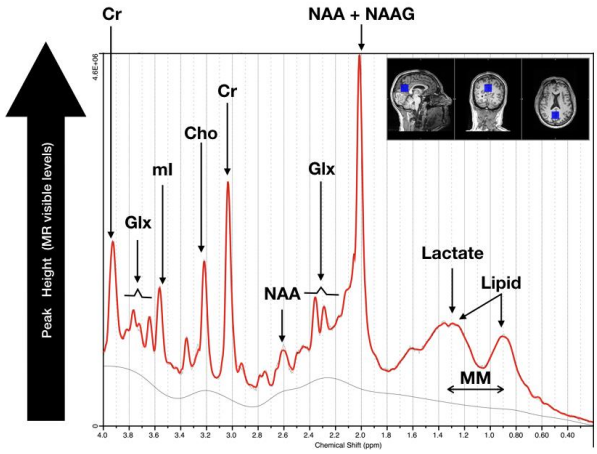

Figure 1 – Example of a MRS spectrum from the posterior cingulate gyrus (PCG) of a healthy human brain at 3T, acquired using PRESS (TE = 30ms, TR = 1500ms) with important metabolite resonances labelled. Inset: MRS voxel location in the PCG. Adapted with permission from Neurospectroscopy: The Past, Present and Future. Carolyn E. Mountford, Peter Stanwell, Alexander Lin, Saadallah Ramadan, and Brian Ross. Chemical Reviews ***2010*** 110 *(5), 3060-3086. DOI: 10.1021/cr900250y. Copyright (2010) American Chemical Society.".* Abbreviations: Cr – Creatine; Glx – Glutamine and Glutamate; mI – myo-inositol; Cho – Choline; NAA – N-Acetylaspartate; NAAG - N-actelyaspartylglutamate; MM – macromolecules.

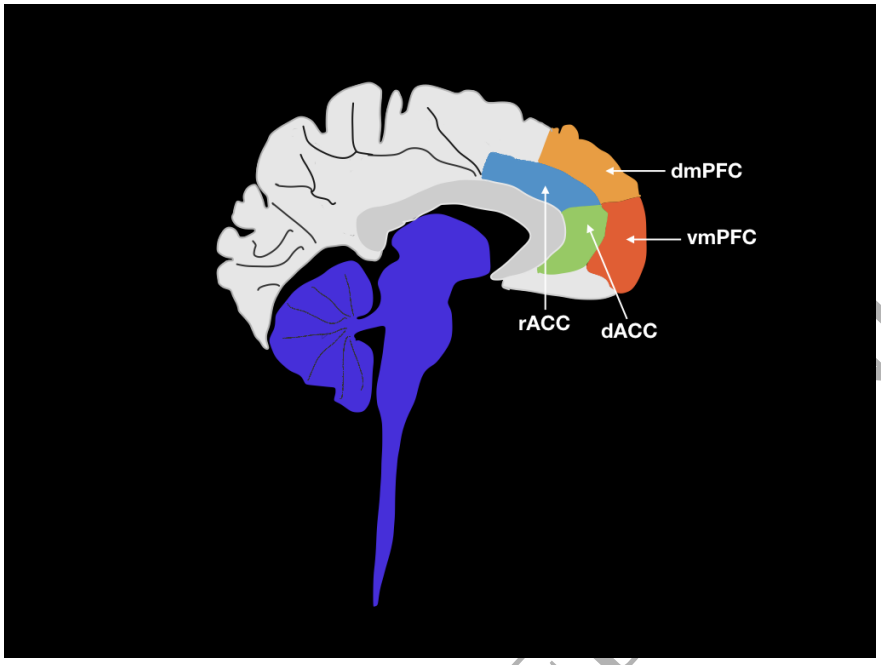

Figure 2 - The medial prefrontal cortex (dmPFC: dorsal medial prefrontal cortex and vmPFC: ventromedial prefrontal cortex) with the anterior cingulate cortex (rACC: rostral anterior cingulate cortex and dACC: dorsal anterior cingulate cortex) are implicated in the pathogenesis of PTSD.